Docket No.: NEB-180

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICANTS: Jack, et al.. EXAMINER: Frank Wei Min Lu

SERIAL NO.: 09/738,444 GROUP: 1655

FILED: December 15, 2000

FOR: Use of Site-Specific Nicking Endonucleases To Create Single-Stranded Regions and Applications Thereof

The Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

RESPONSE A
and
SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

In response to the Office Action mailed on December 19, 2001, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend claim 1 as follows:

A1

1. (amended) A method for creating a target single-stranded region in a double-stranded DNA, comprising:

(a) nicking at least one site bordering the target region within the double-stranded DNA with at least one site-specific nicking endonuclease; and

A1

(b) subjecting the nicked DNA to conditions where the target region is selectively denatured; to create the target single-stranded region in the double-stranded DNA.

Please cancel claims 14-18 and add new claims 22- 30 as follows:

22. (new) A nucleic acid molecule having at least one recognition site and an associated nicking site for a site specific single strand nicking endonuclease, the nucleic acid further comprising a target single strand region in double-stranded DNA formed according to claim 1.

A2

23. (new) A nucleic acid molecule according to claim 22, having a first and a second single strand nicking site wherein the first nicking site is orientated in tandem with respect to the second nicking site.

24. (new) A nucleic acid molecule according to claim 22, having a first and optionally a second single strand nicking site such that the number of nucleotides between the first and the second nicking site or the first nicking site and a terminus of the nucleic acid

molecule determines the length of a single-stranded region in the target single stranded region created according to claim 1.

25. (new) A nucleic acid molecule according to claim 22 having a first single-stranded target region, the first region forming a stable pairing with a second single stranded target region of DNA having a complementary sequence.

26. (new) A nucleic acid molecule according to claim 24, wherein the single-stranded region has a length in the range of 12 to 18 nucleotides.

~~28~~ 27. (new) A nucleic acid molecule according to claim ~~27~~ 26, wherein the single-stranded region has a sequence selected from any of SEQ ID NO:16 through SEQ ID NO:32.

~~29~~ 28. (new) A nucleic acid molecule according to claim ~~27~~ 26, wherein the single-stranded region has a sequence selected from any of SEQ ID NO:35 through SEQ ID NO:49.

~~30~~ 29. (new) A nucleic acid molecule according to claim 22, wherein the recognition site selectively binds the site specific single strand nicking endonuclease, N.BstNBI .

A clean version of the claims showing the amendments made is attached. 37 C.F.R. §1.121(c)(1)(ii).

REMARKS

The Examiner has restricted claims 1-21 into five distinct groups. Applicants elect Group I, claims 1-5 and 14-18 without traverse and cancel claims 6-13 and 19-22 without prejudice.

Claim 1 was objected to because of the following informality: "double-stranded DNA" should be "a double-stranded DNA". Applicants have revised claim 1 to correct this informality. Accordingly, this objection should therefore be withdrawn.

Claims 1 and dependent claims 2-5 and 14-18 were rejected under 35 U.S.C. §112, second paragraph, as being incomplete for omitting essential steps. Applicant thanks the Examiner for suggesting adding a step to remove the objection. Claim 1 has been amended accordingly. Claims 14-18 have been canceled and new claims 22-30 have been added. Support for the new claims can be found page 12, line 20-22, page 13, line 15-page 14, line 3, page 14, lines 4-7, page 15, line 11-12, page 15, line 21-22, page 16, lines 6-7, page 23, lines 27-28, page 26, lines 10-24, page 27, lines 24-page 28, line 3, page 41, line 26-page 42, line 11 and page 43, lines 9-17 .

Rejection under 35 U.S.C.§ 102

The Examiner rejected claims as follows:

(a) Claims 1, 2 and 14-18 are rejected under 35 U.S.C. §102(b) as being anticipated by Xu, et al. (U.S. Patent No. 5,786,195).

(b) Claims 1-5 and 14-16 are rejected under 35 U.S.C. §102(a) as being anticipated by Alland, et al. (Proc. Natl. Acad. Sci. USA, 95:13227-13232 (1998).

(c) Claims 1,17 and 18 are rejected under 35 U.S.C. §102(a) as being anticipated by Wang, et al. (Molecular Biotechnology 15:97-105 (2000)).

According to MPEP ¶ 2131, "to anticipate a claim, the reference must teach every element of the claim". The above cited references do not teach or suggest every element of the claims in the above application. A brief description of the claimed methods as amended is provided below to assist the Examiner in appreciating the differences between what these claimed methods require and what is disclosed in the references.

In claim 1 and dependent claims 2-5, a method is defined for creating a target single stranded region in a double-stranded DNA

that requires nicking with at least one site specific nicking endonuclease and selective denaturation of the double-stranded DNA.

Element (a) of claim 1 states: "nicking at least one site bordering the target region within the double-stranded DNA with at least one site specific nicking endonuclease" The description of the claimed method further states that:

> ..site specific nucleases ...predominantly cleave only one strand of the DNA duplex at defined locations. (page 7, lines 27-29 and page 8, lines 15-17 of the application).

The claimed method further requires a denaturation step in element (b) where the nicked DNA is subjected to denaturation conditions that give rise to target single stranded region in a double-stranded DNA molecule.

According to element (b) of claim 1, a denaturation step alone is not sufficient to achieve the claimed method. Instead, the denaturation must be selective. Claim 1 requires "subjecting the nicked DNA to conditions where the target region is selectively denatured."

The Examiner has recited references that describe cloning methods which rely on restriction endonuclease cleavage of DNA

alone or in combination with PCR. Xu et al. and Alland et al. describe the use of restriction endonuclease digestion of DNA to produce discrete fragments which are blunt ended (AluI) or terminate with 2-4 nucleotide single strand overhangs (PacI, AciI, HinfI). The references fail to disclose the claimed methods for reasons that include the following:

(a) Restriction enzymes cleave double-stranded DNA into discrete fragments without requiring a denaturation step. The product of the restriction endonuclease cleavage may be a blunt ended fragment or a fragment with a short single stranded overhang. The overhang on a restriction fragment has a length which permits dissociation from the cleaved complementary strand without a denaturation step.

Restriction enzymes are bacterial enzymes that recognize 4- to 8-bp sequences called restriction sites and then cleave both DNA strands at this site. (in Molecular Cell Biology 3rd Ed, pg 225, Lodish et al. pub. W.H.Freeman and Company)

Citing Xu et al, the Examiner asserts that the restriction enzymes, AciI and HinfI can be considered as site specific nicking endonucleases since the enzymes provided single stranded overhangs "protruding, cohesive termini". Applicants respectfully assert the opposite. AciI and HinfI cleave double-stranded DNA into distinct fragments by recognizing a single sequence on the DNA and cleaving both strands of the DNA within the single recognized

sequence. The result of the cleavage is two fragments which become physically separated without requiring a denaturation step.

In contrast, site specific endonucleases according to the claimed method do not cleave double-stranded DNA at a single recognition site but instead nick a single strand at a single recognition site bordering a target region. Unlike with restriction endonucleases, the result of single stranded cleavage in non-denaturing conditions is not two fragments. Single stranded DNA is only formed after selective denaturation of the nucleic acid molecule.

(b) Polymerase chain reaction (PCR) requires a denaturation step at 95°C which results in total (non-selective) denaturation of all DNA in a single preparation.

> A typical PCR.....Genomic DNA is digested into large fragments using a restriction enzyme and then is heat denatured into single strands. Two synthetic oligonucleotides complementary to the 3' ends of the DNA segment of interest are added in great excess to the denatured DNA and the temperature is lowered to 50-60°C. The genomic DNA remains denatured because the complementary strands are too low in concentration..... (in Molecular Cell Biology 3rd Ed, pg 254, Lodish et al. pub. W.H.Freeman and Company)

Again citing Xu et al. the Examiner asserts that "the denaturation step in PCR could be considered as step (b) as recited in claims 1 and 2." However, the denaturation step in PCR is

separate and distinct from the claimed method because the denaturation step in PCR is total and non-specific whereas the claimed method requires that "the target region is selectively denatured". The selective denaturation of the claimed method results in partial denaturation of target regions only, the non-target regions remaining double-stranded.

The Examiner has rejected method claims 1-5 as anticipated by Alland et al. because Alland et al. use restriction endonucleases and PCR. The Examiner reports that the reference describes purified cosmid DNA digested with PacI and further digested with AluI restriction endonucleases "and recovering the sequences between 400 and 1,500 bp in length." These fragments are then ligated with XhoI adapters and used for PCR.

The Alland et al. reference teaches away from the present claimed method. AluI and PacI are restriction endonucleases. Although the Examiner asserts otherwise, there is no suggestion or teaching in the reference that these enzymes are site-specific nicking endonucleases nor would such activity be useful for the cited purpose of the reference. Moreover, the Examiner has given no basis for the contradictory assertion concerning PacI and AluI activity on page 10 of the Office Action.

The Examiner has provided the New England Biolabs' catalog describing the PacI cleavage site which occurs on double-stranded DNA. As described above, the enzyme cleaves both strands to provide two fragments, each fragment having a single strand 3' dinucleotide overhang. No selective denaturation is described nor indeed would be desirable to form the two fragments. The reported use of XhoI adapters is for creating a priming site for PCR amplification and produces blunt-end fragments later used in detection protocols. The use of XhoI adapters has no relevance to the present claimed method. The denaturation step in PCR recited by the Examiner has been addressed above with respect to Xu et al. Denaturation during PCR is distinct from the present claimed method for reasons that include non-specificity.

The Examiner has rejected claims 1, 17 and 18 as anticipated by Wang et al. citing nicking and denaturation steps. The Wang, et al. reference describes a use of the site-specific nicking endonuclease N.BstNBI to introduce a single nick into a covalently closed circular molecule. The resulting molecule is denatured in entirety, resulting in circular (the unnicked strand) and linear (the nicked strand) single-stranded DNAs.

The Wang et al. method is directed to producing mismatched-DNA substrates. (page 97). The cited method uses nicking enzymes for initiating mismatch repair. The method includes mixing whole

denatured linearized PUC19HE with whole denatured nicked pUC19XE. Reannealing is permitted and mismatch oligoduplexes formed. (pg. 101). There is no suggestion or teaching that selective denaturation of target DNA be undertaken so as to create a single stranded region in a double-stranded DNA.

The reference teaches away from the present claimed method because the present claimed invention is directed to creating single-stranded regions in double-stranded molecules. The claim states "...in double-stranded DNA..." rather than "...from double-stranded DNA...." (claim 1) The claimed method requires selective denaturation of only the target region.

In summary, the present claims are not anticipated by the cited prior art because none of the cited references teach every element of claim 1. Where independent claim 1 is found to be novel and the method patentable, claims dependent on claim 1 will be found to be novel and patentable. Applicants have shown above why the cited references are distinct from the claimed method of claim 1 and respectfully request that the rejection be reversed and all claims allowed.